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Form PTO-1390 U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE (REV 10-95) TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371		ATTORNEY'S DOCKET NUMBER 702-011584
		U.S. APPLICATION NO. (If known, see 37 CFR 1.5) 09/937466
INTERNATIONAL APPLICATION NO. PCT/NL00/00196	INTERNATIONAL FILING DATE 23.03.00 (23 March 2000)	PRIORITY DATES CLAIMED 26.03.99 (26 March 1999)
TITLE OF INVENTION METHOD AND COMPOSITION FOR CONTROLLING LICE		
APPLICANT(S) FOR DO/EO/US Klaas D. KUSSENDRAGER and Petrus M. VAN HAAREN		
<p>Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:</p> <ol style="list-style-type: none"> <input checked="" type="checkbox"/> This is a FIRST submission of items concerning a filing under 35 U.S.C. 371. <input type="checkbox"/> This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371. <input checked="" type="checkbox"/> This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1). <input checked="" type="checkbox"/> A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date. <input checked="" type="checkbox"/> A copy of the International Application as filed (35 U.S.C. 371(c)(2)) <ol style="list-style-type: none"> <input type="checkbox"/> is transmitted herewith (required only if not transmitted by the International Bureau). <input checked="" type="checkbox"/> has been transmitted by the International Bureau. <input type="checkbox"/> is not required, as the application was filed in the United States Receiving Office (RO/US). <input type="checkbox"/> A translation of the International Application into English (35 U.S.C. 371(c)(2)). <input checked="" type="checkbox"/> Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3)) <ol style="list-style-type: none"> <input type="checkbox"/> are transmitted herewith (required only if not transmitted by the International Bureau). <input type="checkbox"/> have been transmitted by the International Bureau. <input type="checkbox"/> have not been made; however, the time limit for making such amendments has NOT expired. <input checked="" type="checkbox"/> have not been made and will not be made. <input type="checkbox"/> A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)). <input type="checkbox"/> An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)). <input type="checkbox"/> A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)). <p>Items 11. to 16. below concern document(s) or information included:</p> <ol style="list-style-type: none"> <input type="checkbox"/> An Information Disclosure Statement under 37 CFR 1.97 and 1.98. <input type="checkbox"/> An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included. <input checked="" type="checkbox"/> A FIRST preliminary amendment. <input type="checkbox"/> A SECOND or SUBSEQUENT preliminary amendment. <input type="checkbox"/> A substitute specification. <input type="checkbox"/> A change of power of attorney and/or address letter. <input checked="" type="checkbox"/> Other items or information: <ol style="list-style-type: none"> WO 00/57704-Front Page with Abstract, specification, and claims (16 pp.) International Search Report (2 pp.) International Preliminary Examination Report (6 pp.) 		

U.S. APPLICATION NO. (if known to 37 CFR 1.492(a)(1)-(5)) 09/937466		INTERNATIONAL APPLICATION NO. PCT/NL00/00196		ATTORNEY'S DOCKET NUMBER 702-011584	
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<p>17. <input checked="" type="checkbox"/> The following fees are submitted:</p> <p>BASIC NATIONAL FEE (37 CFR 1.492(a)(1)-(5)):</p> <p>Search Report has been prepared by the EPO or JPO..... \$860.00</p> <p>International preliminary examination fee paid to USPTO (37 CFR 1.482)..... \$690.00</p> <p>No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2))..... \$710.00</p> <p>Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO..... \$1000.00</p> <p>International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4)..... \$100.00</p>				<p>CALCULATIONS PTO USE ONLY</p>	
ENTER APPROPRIATE BASIC FEE AMOUNT =				\$ 860.00	
Surcharge of \$130.00 for furnishing the oath or declaration later than <input type="checkbox"/> 20 <input checked="" type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(e)).				\$ 130.00	
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	17 - 20	0	X \$18.00	\$ 0.00	
Independent claims	3 - 3 =	0	X \$80.00	\$ 0.00	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ \$270.00	\$ 0.00	
TOTAL OF ABOVE CALCULATIONS =				\$ 990.00	
Reduction of 1/2 for filing by small entity, if applicable.				\$ 0.00	
SUBTOTAL =				\$ 990.00	
Processing fee of \$130.00 for furnishing the English translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(f)).				\$ 0.00	
TOTAL NATIONAL FEE =				\$ 990.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00 per property				\$ 0.00	
TOTAL FEES ENCLOSED =				\$ 990.00	
				Amount to be: Refunded	\$
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a. ☒ A check in the amount of **\$ 990.00** to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. _____ in the amount of \$ _____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Assistant Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 23-0650. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

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09/937466

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PATENT APPLICATION/PCT
Attorney Docket No. 702-011584

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of :

Klaas D. KUSSENDRAGER : METHOD AND COMPOSITION FOR
Petrus M. VAN HAAREN : CONTROLLING LICE

International Application :
No. PCT/NL00/00196 :

International Filing Date :
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26 March 1999 :

Serial No. Not Yet Assigned :

Filed Concurrently Herewith :

Pittsburgh, Pennsylvania
September 25, 2001

PRELIMINARY AMENDMENT

Box PCT
Commissioner for Patents
Washington, D.C. 20231

Sir:

Prior to initial examination, please amend the above-identified patent application as follows:

IN THE SPECIFICATION:

Please insert section headings and insert specification paragraphs as follows.

On page 1, after the title, at line 2, please insert the following section heading:

BACKGROUND OF THE INVENTION

Before the paragraph beginning at page 2, line 13, please insert the following section heading:

SUMMARY OF THE INVENTION

Before the paragraph beginning at page 2, line 29, please insert the following section heading and specification paragraphs:

DETAILED DESCRIPTION OF THE INVENTION

This is achieved by the invention with a method for controlling lice on an organism, comprising of treating the organism with a composition which at least consists of:

- a) lactoperoxidase;
- b) thiocyanate; and/or
- c) iodide; and
- d) a hydrogen peroxide source.

Although this method is particularly suitable for controlling sea lice, it can also be employed to control other lice, such as aphids on plants, lice on animals, such as head lice in people.

IN THE CLAIMS:

Please cancel claims 1-20 rewrite them as new claims 21-37 as follows:

21. A method for controlling lice on an organism, comprising of treating the organism with a composition at least consisting of:

- a) lactoperoxidase;
- b) thiocyanate and/or iodide; and
- c) a hydrogen peroxide source.

22. The method as claimed in claim 21, wherein the hydrogen peroxide source is hydrogen peroxide itself or a system of glucose oxidase and glucose whereby hydrogen peroxide can be generated.

23. The method as claimed in claim 21, wherein the lice are sea lice and the organism is a fish or crustacean.

24. The method as claimed in claim 21, wherein the composition is added to the water in which the fish are kept.

25. The method as claimed in claim 21, wherein the lice are aphids and the organism is a plant.

26. The method as claimed in claim 21, wherein the lice are located on an animal.

27. The method as claimed in claim 21, wherein the lice are head lice and the organism is a human.

28. The method as claimed in claim 21, wherein the concentration of lactoperoxidase amounts to 0.5 to 20 mg/l, together with at least about 10 mg/l hydrogen peroxide, and at least about 2.5 mg/l thiocyanate or at least about 5 mg/l iodide.

29. The method as claimed in claim 21, wherein the concentration of lactoperoxidase amounts to 1 to 10 mg/l, together with at least about 50 mg/l hydrogen peroxide, and at least about 5 mg/l thiocyanate or at least about 20 mg/l iodide.

30. The method as claimed in claim 21, wherein the concentration of lactoperoxidase amounts to 2.5 to 7.5 mg/l, together with at least about 100 mg/l hydrogen peroxide and at least about 10 mg/l (maximum 100 mg/l) thiocyanate or at least about 20 mg/l, maximum 100 mg/l, iodide.

31. The method as claimed in claim 21, wherein the concentration of lactoperoxidase amounts to 5 mg/l together with at least about 100 mg/l hydrogen peroxide and at least about 10 mg/l (maximum 100 mg/l) thiocyanate or at least about 20 mg/l, maximum 100 mg/l, iodide.

32. A composition for controlling lice on an organism, comprising:

- a) lactoperoxidase in a quantity of 0.5 to 50 mg/l;
- b) hydrogen peroxide in a quantity of at least 10 mg/l;
- c) thiocyanate in a quantity of at least about 2.5 mg/l; and
- d) iodide in a quantity of at least about 5 mg/l,

wherein all quantities of active substance refer to the concentration of the active substance with which the lice come into contact.

33. The composition as claimed in claim 32, comprising: 50 mg/l lactoperoxidase, 10 mg/l thiocyanate, 30 mg/l iodide and 100 mg/l hydrogen peroxide.

34. A kit for controlling lice on an organism, which kit comprises at least two components, wherein the first component is formed by a composition comprising lactoperoxidase in a quantity resulting in a concentration with which the lice come into contact of 0.5 to 20 mg/l, thiocyanate in a quantity resulting in a concentration with which the lice come into contact of at least about 2.5 mg/l and/or iodide in a quantity resulting in a concentration with which the lice come into contact of at least about 5 mg/l, and the second component is formed by a solution of hydrogen peroxide in a quantity resulting in a concentration with which the lice come into contact of at least 10 mg/l.

35. The kit as claimed in claim 33, wherein the first component consists of lactoperoxidase in a quantity resulting in a concentration with which the lice come into contact of about 5 mg/l, thiocyanate in a quantity resulting in a concentration with which the lice come into contact of about 10 mg/l and/or iodide in a quantity resulting in a

concentration with which the lice come into contact of about 30 mg/l, and the second component is formed by a solution of hydrogen peroxide in a quantity resulting in a concentration with which the lice come into contact of 100 mg/l.

36. The kit as claimed in claim 35, wherein the first component is a concentrated liquid.

37. The kit as claimed in claim 35, wherein the first component has a solid form.

IN THE ABSTRACT:

After the claims, please insert a page containing the Abstract Of The Disclosure, which is attached hereto as a separately typed page.

REMARKS

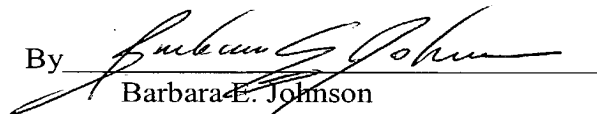
The specification and claim amendments have been made in order to conform this patent application to customary United States patent practice.

Examination and allowance of pending claims 21-37 are respectfully requested.

Respectfully submitted,

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METHOD AND COMPOSITION FOR CONTROLLING LICE

ABSTRACT OF THE INVENTION

The invention relates to a method for controlling lice on an organism, comprising of treating the organism with a composition at least consisting of: a) lactoperoxidase; b) thiocyanate and/or iodide; and c) a hydrogen peroxide source. The method is particularly suitable for controlling sea lice in fish or crustaceans, but can also be applied against aphids on plants and head lice on humans. The invention further relates to a composition and kit for use in the method.

METHOD AND COMPOSITION FOR CONTROLLING LICE

The present invention relates to a method and device
5 for controlling lice, in particular sea lice.

Sea louse is the general name of parasitic oar-
footed crustaceans (copepods), which are found at
(marine) water culture production locations where fish
are farmed. In Northern Europe and particularly in
10 Scotland and Norway sea lice are already the most
significant threat to production locations of sea fish
and the problem only continues to grow. Damage and death
of fish due to sea louse infections are an important cost
overhead in aquaculture. The problem also occurs in
15 seawater and brackish water fish farms and shrimp ponds
in South-East Asia, particularly in Thailand, the
Philippines and Indonesia.

The parasitic sea louse copepods belong to the
family of the Caligidae, comprising 23 genera and 200
20 species. The most important genera are Lepeophtheirus,
Caligus and Pseudocaligus, because they can result in
high mortality. In Northern Europe the most important
parasite is Lepeophtheirus salmonis and to a lesser
degree Caligus elongatus. Both belong to the Caligidae
25 and are ectoparasites on salmonoids. The life cycle of
L. salmonis comprises 10 stages, of which the 4 chalimus
stages infect the salmon. They attach themselves to the
fish with claw-shaped antennae, penetrate the skin of the
fish and thus cause skin lesions and large open wounds.
30 Secondary bacterial and fungal infections are
subsequently often the cause of death of the fish.

In order to control the sea lice use is often made
of hydrogen peroxide, which is added to a treatment bath
in a quantity of about 1500 mg/l (see for instance US-
35 5,313,911 in the name of Eka Nobel AB). However, both the
large volumes of hydrogen peroxide and the limited
activity and toxicity for the fish do not make this an
ideal method.

Bath treatments are further also applied with different types of pesticide such as Cypermethrin™, Nuvan™ (active substance dichlorvos), Pyrethrum™ and Dipterex™ (active substance trichlorophen). These substances can only be used under heavy restrictions and have great drawbacks. They are not only toxic for the lice, but also harmful to the fish and the environment. Residues of the substances moreover accumulate in the fish and thus form an indirect risk to the consumer.

10 Handling of the substances also involves risks. Finally, these substances are not active against every stage of development of the sea lice.

In the light of the above, it is the object of the present invention to provide an effective, natural and environmentally-friendly system with which the lice can be controlled without too many drawbacks for the fish.

This is achieved by the invention with a method for controlling lice on an organism, comprising of treating the organism with a composition which at least consists of:

- a) lactoperoxidase;
- b) thiocyanate; and/or
- c) iodide; and
- d) a hydrogen peroxide source.

25 Although this method is particularly suitable for controlling sea lice, it can also be employed to control other lice, such as aphids on plants, lice on animals, such as head lice in people.

The methods of administration in controlling other types of lice are of course not the same as in controlling sea lice. In the latter case the agent is added to water in which the fish are accommodated for a longer or shorter time or in which they are immersed. Controlling lice on plants can for instance be done by spraying, while treatment of head louse can take place by rinsing, rubbing-in or spraying.

The composition consisting of lactoperoxidase, thiocyanate and/or iodide and hydrogen peroxide is most

effective when the concentration thereof with which the lice come into contact amounts for lactoperoxidase (LP) to 0.5 to 20 mg/l, preferably 1 to 10 mg/l, more preferably 2.5 to 7.5 mg/l and most preferably about 5 mg/l, for hydrogen peroxide to at least 10, preferably at least 50, more preferably at least 100 mg/l, for thiocyanate (SCN⁻) to at least about 2.5 mg/l, preferably at least about 5 mg/l, more preferably at least about 10 mg/l, but a maximum of 100 mg/l, and for iodide (I⁻) to at least about 5 mg/l, preferably at least about 30 mg/l, but a maximum of 100 mg/l.

When reference is made in this application to "concentration with which the lice come into contact", this is intended to mean the concentration which is present in the treatment bath in the case of sea lice, or in the spray or other means of application in the case of other lice. "Composition" is likewise understood to mean that in which the concentrations are equal to the treatment concentrations.

Such a composition for controlling lice on an organism therefore comprises the following active substances:

- a) lactoperoxidase (LP) in a quantity of 0.5 to 20 mg/l, preferably 1 to 10 mg/l, more preferably 2.5 to 7.5 mg/l and most preferably about 5 mg/l;
- b) hydrogen peroxide in a quantity of at least 10, preferably at least 50, more preferably at least 100 mg/l;
- c) thiocyanate (SCN⁻) in a quantity of at least about 2.5 mg/l, preferably at least about 5 mg/l, more preferably at least about 10 mg/l, but a maximum of 100 mg/l, and
- d) iodide (I⁻) in a quantity of at least about 5 mg/l, preferably at least about 20 mg/l, but a maximum of 100 mg/l. In a preferred embodiment the composition according to the invention comprises: 5 mg/l lactoperoxidase, 10 mg/l thiocyanate, 30 mg/l iodide and 100 mg/l hydrogen peroxide.

This composition can in turn be manufactured from a kit consisting of at least two components.

The components of the kit are at least two components, wherein the first component comprises
5 lactoperoxidase, thiocyanate and/or iodide and the second component hydrogen peroxide. Supplying hydrogen peroxide separately prevents lactoperoxidase already becoming active in the packaging. The activity of the final composition could thereby be reduced. A kit may however
10 also consist of more than two components, wherein in addition to the separate hydrogen peroxide the other constituents are also supplied separately or in pairs of two. The components can each individually be in liquid or solid form. Particularly the component consisting of
15 lactoperoxidase, thiocyanate and/or iodide also forms part of the invention.

A particularly advantageous embodiment of the kit comprises at least two components, wherein the first component is formed by a composition comprising
20 lactoperoxidase in a quantity resulting in a concentration with which the lice come into contact of 0.5 to 20 mg/l, preferably 1 to 10 mg/l, more preferably 2.5 to 7.5 mg/l and most preferably about 5 mg/l, thiocyanate in a quantity resulting in a concentration
25 with which the lice come into contact of at least about 2.5 mg/l, preferably at least about 5 mg/l, more preferably at least about 10 mg/l, but a maximum of 100 mg/l, and/or iodide in a quantity resulting in a concentration with which the lice come into contact of at
30 least about 5 mg/l, preferably at least about 20 mg/l, but a maximum of 100 mg/l, and the second component is formed by a solution of hydrogen peroxide in a quantity resulting in a concentration with which the lice come into contact of at least 10, preferably at least 50, more
35 preferably at least 100 mg/l. Particularly recommended is a kit wherein the first component consists of lactoperoxidase in a quantity resulting in a concentration with which the lice come into contact of

about 5 mg/l, thiocyanate in a quantity resulting in a concentration with which the lice come into contact of about 10 mg/l and/or iodide in a quantity resulting in a concentration with which the lice come into contact of about 30 mg/l, and the second component is formed by a solution of hydrogen peroxide in a quantity resulting in a concentration with which the lice come into contact of 100 mg/l.

The composition can also be formed from a concentrated composition or a solid composition which contains all constituents. The desired treatment concentrations then result by adding this concentrated liquid or solid composition to water.

It is recommended to prepare the treatment bath prior to the fish being placed therein. This prevents damage to the fish occurring due to locally high concentrations of the different constituents which have not yet dissolved or are not yet well distributed through the water.

In order to safeguard the health of the fish as much as possible, the treatment time is preferably kept as short as possible. Treatment times between 5 and 60 minutes are recommended. Although the agent according to the invention is much less toxic than the high concentrations of hydrogen peroxide or pesticides used heretofore, it is nevertheless recommended to limit the contact between fish and active substances as much as possible. The treatment may optionally be repeated at a later stage in the case control is not complete.

The invention further relates to the use of the composition, kit or components thereof for controlling lice in general and sea lice in particular. The invention also comprises the use of the kit or the components therefrom for manufacturing the composition.

The invention will be further illustrated with reference to the examples following below. The composition according to the invention is herein designated as "LP system".

EXAMPLESEXAMPLE 1In vitro tests with lactoperoxidase (LP) systems against sea louse

5

Egg sacs of the sea louse Lepeophteirus salmonis were collected and incubated for 5 days at 15°C in water with a salinity of 3.4‰ per weight. During this time the oar-footed crustaceans developed into healthy specimens.

10 One or more of the components lactoperoxidase, iodide, thiocyanate and hydrogen peroxide were dissolved in seawater sterilized using an ozone treatment and filtration. About 200 copepodids per litre were subsequently added to each of these solutions and
15 incubated for 20 minutes. The copepodids were then filtered off, washed and placed once again in clean seawater. The percentage of surviving copepodids was determined after 1 hour.

The composition of the solutions and the survival of
20 the copepodids therein are shown in tables 1 and 2.

Table 1 Effect of individual components

	Blank	I ⁻ Only	SCN ⁻ Only	H ₂ O ₂ Only	LP Only	LP Only
Lactoperoxidase, mg/l	0	0	0	0	10	20
K-iodide, mg/l	0	30	0	0	0	0
K-thiocyanate, mg/l	0	0	10	0	0	0
H ₂ O ₂ , mg/l	0	0	0	200	0	0
% survival after 1 hour	95	88	86	93	90	69

10 Table 2 Effect of LP systems

Lactoperoxidase, mg/l	0	2.5	5	10	10	10	20	20
K-iodide, mg/l	0	30	30	30	30	30	30	30
K-thiocyanate, mg/l	0	10	10	10	10	10	10	10
H ₂ O ₂ , mg/l	0	100	100	10	50	100	100	200
% survival after 1 hour	95	73	39	31	6	0	0	0

From tables 1 and 2 can be seen that the individual components have hardly any effect on the sea lice, but that the combination thereof in the LP system according to the invention does so.

EXAMPLE 2

25 Sensitivity of fish to LP systems

Young salmon with an average weight of about 50 g. were exposed to solutions (in seawater) of the individual components and to complete LP systems. The table below shows the concentration.

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components group	control	1	2	3	4	5	6
Lactoperoxidase mg/l	0	10	0	0	5	2.5	1
K-thiocyanate mg/l	0	0	10	0	5	2.5	2.5
K-iodide mg/l	0	0	0	30	5	7.5	7.5
H ₂ O ₂ mg/l	0	0	0	0	100	100	100

The transfer of young salmon to a new environment will in any case cause stress phenomena, such as a slightly increased gill cover activity and agitated swimming on the surface. Calm is virtually restored after about 30 minutes.

These phenomena were observed in both the control and solutions of the individual components, wherein there was hardly any difference, or none at all, between the control and individual components.

The stress reactions with complete LP systems were clearly higher, at the lowest concentration (1 mg/l LP) agitated swimming behaviour and a moderately increased gill cover activity was still present after 30 minutes. Only after 60 minutes was calm restored.

At a concentration of 2.5 mg/l LP these phenomena were more pronounced but still acceptable.

At a concentration of 5 ppm LP stress phenomena were even more severe. After 60 minutes the majority of the fish was still lethargic and swimming in uncoordinated manner. There were no fatalities however.

A test with larger salmon (about 500 g) and a system with 5 mg/l LP showed that these fish were hardly affected by the treatment.

EXAMPLE 3

In vivo study of the effect of LP systems on fish which "infected" with sea lice

In this example the effect of an LP system with 2.5 mg/l LP, 2.5 mg/l KSCN, 7.5 mg/l KI and 100 mg/l H₂O₂ is

studied in a situation in which young Atlantic salmon were "infected" with sea lice of L. salmonis.

1. Method

5 In four tanks of 1m³ each, 40 young salmon of about 50g in their second year of life were kept per tank in seawater treated with ozone (>750 Mv) and filtered by carbon at ambient temperature (14°C ± 1°C). Per tank the fish were brought into contact with 1000 copepodids of L.
10 salmonis for 1.5 hours and the lice were allowed to develop to pre-adult stages.

Counts were carried out prior to the treatment and it was determined that all tanks contained sufficient pre-adult stage lice. The treatments were then started.

15 Two of the tanks (1 and 3) were treated with the LP system according to the invention (2.5 mg/l LP, 2.5 mg/l KSCN, 7.5 mg/l KI and 100 mg/l H₂O₂) for 20 minutes. Tanks 2 and 4 received an identical treatment with seawater. The temperature of the seawater was 15°C and it contained
20 more than 8 mg/l oxygen. Samples were assessed 1, 24 and 48 hours after treatment. Lice levels on the fish were recorded and compared with the levels before the treatment making use of Student's t-test.

25 2. Results

The results of the lice counts are shown in the table below. The lice counts are expressed per fish and are average values of 10 fish.

30

35

Table 9

Sea lice counts: average values per fish (n=10)

	before treatment	after treatment, 1 hour	after treatment, 24 hours	after treatment, 48 hours
Tank 1 (LP-s)	4.0 (SEM = 0.494)	3.3 (SEM = 0.60)	2.5 (SEM = 0.64)	1.5 (SEM = 0.5)
Tank 2 (control)	4.8 (SEM = 0.629)	4.4 (SEM = 0.56)	4.2 (SEM = 0.61)	3.4 (SEM = 0.4)
Tank 3 (LP-s)	4.7 (SEM = 0.731)	4.0 (SEM = 0.56)	2.1 (SEM = 0.41)	2.2 (SEM = 0.2)
Tank 4 (control)	3.5 (SEM = 0.401)	5.1 (SEM = 0.74)	3.7 (SEM = 0.94)	3.8 (SEM = 1.08)

Lice levels

1 hour after the treatment there was no significant reduction in lice levels in any of the groups. After 24 hours both treated groups had significantly fewer lice. 48 hours after the treatment there was a reduction of 5 respectively 63% and 53% ($p < 0.01$). There was no significant reduction in lice levels in untreated control groups.

Behaviour of the fish

In the eleventh minute during the treatment the fish displayed some agitation with an increase to rapid swimming and jumping activity at 15 minutes. At 19 minutes some fish were at the point of death and only recovered after the tank had been flushed. There were no fatalities.

15 The treatment with an LP system consisting of 2.5 mg/l LP, 2.5 mg/l KSCN, 7.5 mg/l KI and 100 mg/l H_2O_2 for 20 minutes was on average 58% effective against the sea lice. There appeared to be some effect on the fish, but this was not fatal. Because small fish were treated here
20 at a high seawater temperature, this test was performed under the worst possible conditions. Larger fish at a lower temperature will be more resistant to the effects of an LP system.

CLAIMS

1. Method for controlling lice on an organism, comprising of treating the organism with a composition at least consisting of:

- a) lactoperoxidase;
- 5 b) thiocyanate; and/or
- c) iodide; and
- d) a hydrogen peroxide source.

2. Method as claimed in claim 1, characterized in that the hydrogen peroxide source is hydrogen peroxide
10 itself or a system of glucose oxidase and glucose whereby hydrogen peroxide can be generated.

3. Method as claimed in claims 1 and 2, characterized in that the lice are sea lice and the organism is a fish or crustacean.

15 4. Method as claimed in claim 3, characterized in that the composition is added to the water in which the fish are kept.

4. Method as claimed in claims 1 and 2, characterized in that the lice are aphids and the
20 organism is a plant.

5. Method as claimed in claims 1 and 2, characterized in that the lice are located on an animal.

6. Method as claimed in claims 1 and 2, characterized in that the lice are head lice and the
25 organism is a human.

7. Method as claimed in claims 1-6, characterized in that the concentration of lactoperoxidase (LP) with which the lice come into contact amounts to 0.5 to 20 mg/l, preferably 1 to 10 mg/l, more preferably 2.5 to 7.5 mg/l
30 and most preferably about 5 mg/l.

8. Method as claimed in claims 1-7, characterized in that the concentration of hydrogen peroxide with which the lice come into contact amounts to at least 10, preferably at least 50, more preferably at least 100
35 mg/l.

9. Method as claimed in claims 1-8, characterized in that the concentration of thiocyanate (SCN^-) with which the lice come into contact amounts to at least about 2.5 mg/l, preferably at least about 5 mg/l, more preferably at least about 10 mg/l, but a maximum of 100 mg/l.

10. Method as claimed in claims 1-9, characterized in that the concentration iodide (I^-) with which the lice come into contact amounts to at least about 5 mg/l, preferably at least about 20 mg/l, but a maximum of 100 mg/l.

11. Composition for controlling lice on an organism, comprising:

a) lactoperoxidase (LP) in a quantity of 0.5 to 20 mg/l, preferably 1 to 10 mg/l, more preferably 2.5 to 7.5 mg/l and most preferably about 5 mg/l;

b) hydrogen peroxide in a quantity of at least 10, preferably at least 50, more preferably at least 100 mg/l;

c) thiocyanate (SCN^-) in a quantity of at least about 2.5 mg/l, preferably at least about 5 mg/l, more preferably at least about 10 mg/l, but a maximum of 100 mg/l; and

d) iodide (I^-) in a quantity of at least about 5 mg/l, preferably at least about 20 mg/l, but a maximum of 100 mg/l,

wherein all quantities of active substance refer to the concentration of the active substance with which the lice come into contact.

12. Composition as claimed in claim 11, comprising: 50 mg/l lactoperoxidase, 10 mg/l thiocyanate, 30 mg/l iodide and 100 mg/l hydrogen peroxide.

13. Kit for controlling lice on an organism, which kit comprises at least two components, wherein the first component is formed by a composition comprising lactoperoxidase in a quantity resulting in a concentration with which the lice come into contact of 0.5 to 20 mg/l, preferably 1 to 10 mg/l, more preferably 2.5 to 7.5 mg/l and most preferably about 5 mg/l,

thiocyanate in a quantity resulting in a concentration with which the lice come into contact of at least about 2.5 mg/l, preferably at least about 5 mg/l, more preferably at least about 10 mg/l, but a maximum of 100 mg/l, and/or iodide in a quantity resulting in a concentration with which the lice come into contact of at least about 5 mg/l, preferably at least about 30 mg/l, but a maximum of 100 mg/l, and the second component is formed by a solution of hydrogen peroxide in a quantity resulting in a concentration with which the lice come into contact of at least 10, preferably at least 50, more preferably at least 100 mg/l.

14. Kit as claimed in claim 12, characterized in that the first component consists of lactoperoxidase in a quantity resulting in a concentration with which the lice come into contact of about 5 mg/l, thiocyanate in a quantity resulting in a concentration with which the lice come into contact of about 10 mg/l and/or iodide in a quantity resulting in a concentration with which the lice come into contact of about 30 mg/l, and the second component is formed by a solution of hydrogen peroxide in a quantity resulting in a concentration with which the lice come into contact of 100 mg/l.

15. Kit as claimed in claims 13 and 14, characterized in that the first component is a concentrated liquid.

16. Kit as claimed in claims 13 and 14, characterized in that the first component has a solid form.

17. Component for use in a kit as claimed in claims 13-16, comprising lactoperoxidase, thiocyanate and/or iodide in a quantity resulting in a concentration with which the lice come into contact as stated in claim 13 or 14.

18. Use of a composition as claimed in claims 11 and 12 for controlling lice on an organism.

19. Use of the kit as claimed in claims 13-16 for manufacturing a composition as claimed in claim 11 or 12.

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20. Use of a component as claimed in claim 17 in a kit as claimed in claims 13-16.

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(21) International Application Number: PCT/NL00/00196 (22) International Filing Date: 23 March 2000 (23.03.00) (30) Priority Data: 1011681 26 March 1999 (26.03.99) NL (71) Applicant (for all designated States except US): CAMPINA MELKUNIE B.V. [NL/NL]; Hogeweg 9, NL-5301 LB Zaltbommel (NL). (72) Inventors; and (75) Inventors/Applicants (for US only): KUSSENDRAGER, Klaas, Daniël [NL/NL]; Eksterdonk 17, NL-5467 DN Veghel (NL). VAN HAAREN, Petrus, Martinus [NL/NL]; Dr. Van de Voortsingel 5, NL-5461 AM Veghel (NL). (74) Agent: VAN SOMEREN, Petronella, Francisca, Hendrika, Maria; Arnold & Siedsma, Sweelinckplein 1, NL-2517 GK The Hague (NL).	(81) Designated States: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published With international search report. In English translation (filed in Dutch).	
(54) Title: METHOD AND COMPOSITION FOR CONTROLLING LICE (57) Abstract <p>The invention relates to a method for controlling lice on an organism, comprising of treating the organism with a composition at least consisting of: a) lactoperoxidase; b) thiocyanate; and/or c) iodide; and d) a hydrogen peroxide source. The method is particularly suitable for controlling sea lice in fish or crustaceans, but can also be applied against aphids on plants and head lice on humans. The invention further relates to a composition and kit for use in the method.</p>		

Declaration and Power of Attorney For Patent Application

English Language Declaration

As a below named inventor, I hereby declare that: 1) Kussendrager, Klaas Daniel
2) Van Haaren, Petrus Martinus

My residence, post office address and citizenship are as stated below next to my name.

1) Eksterdonk 17, NL-5467 DN VEGHEL, The Netherlands
2) Dr. Van de Voortsingel 5, NL-5461 AM VEGHEL, The Netherlands
I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

Method and composition for controlling lice
the specification of which

(check one)

☐ is attached hereto.

☒ was filed on 25 Sept. 2001 as

Application Serial No. 09/937,466

and was amended on _____
(if applicable)

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Prior Foreign Application(s)

Priority Claimed

<u>1011681</u>	<u>NL</u>	<u>26 March 1999</u>
(Number)	(Country)	(Day/Month/Year Filed)
<u> </u>	<u> </u>	<u> </u>
(Number)	(Country)	(Day/Month/Year Filed)
<u> </u>	<u> </u>	<u> </u>
(Number)	(Country)	(Day/Month/Year Filed)

<input checked="" type="checkbox"/>	<input type="checkbox"/>
Yes	No
<input type="checkbox"/>	<input type="checkbox"/>
Yes	No
<input type="checkbox"/>	<input type="checkbox"/>
Yes	No

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

(Application Serial No.)

(Filing Date)

(Status)
(patented, pending, abandoned)

(Application Serial No.)

(Filing Date)

(Status)
(patented, pending, abandoned)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith. (list name and registration number)

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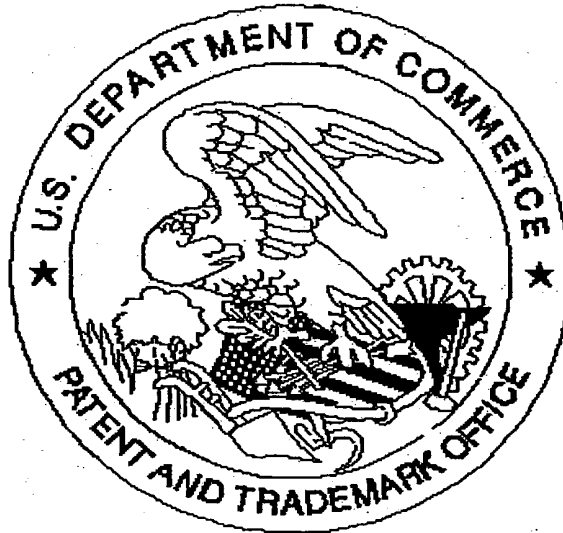
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